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$(GTG)_5$-PCR analysis and 16S rRNA sequencing of bacteria from Sarawak aquaculture environment

Kathleen, M. M., Samuel, L., Felecia, C., Ng K. H., Lesley, M. B. and Kasing, A.

Department of Molecular Biology, Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia

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Abstract

$(GTG)_5$ PCR is a type of repetitive extragenic palindromic (rep)-PCR which amplifies the $(GTG)_5$ repetitive element that lays throughout the bacterial genome. In this study, fifty, thirty-nine and forty-nine unknown bacteria were isolated from aquaculture farms in Miri, Limbang and Lundu, respectively. $(GTG)_5$ PCR was used to screen for clonal diversity among the isolates according to sampling sites. Banding profiles obtained from electrophoresed $(GTG)_5$ PCR products were analyzed by RAPDistance Software to generate a dendrogram of neighbor joining tree (NJT) format. Based on the constructed dendrogram, representative isolates were selected for further identification. Conserved 16S rRNA region of the selected bacteria isolates were amplified and purified DNA products were sequenced. $(GTG)_5$ PCR is useful in differentiation of unknown bacterial isolates and 16S rRNA analysis species identity of the bacteria in Sarawak aquaculture environment. The high diversity of bacteria in aquaculture environment may be caused by contamination from various sources.

Introduction

Aquaculture sector is one of the important and rapidly growing sectors in Malaysia. Large number of bacteria from highly diverse bacterial species could be isolated from the aquaculture pond and its environment. Presence of a large number of bacteria in the pond indicates the presence of high nutrient level in the water. The number and species of freshwater microbiota depends on the availability of light and oxygen (Tortora *et al*., 2007). Based on previous study done by Apun *et al.* (1999), fish intestine contains the most number of bacteria from different species. Among the commonly found bacterial species in aquaculture pond and its environment were *Aeromonas*, *Escherichia*, *Enterobacter*, *Klebsiella*, *Pseudomonas*, *Vibrio*, *Bacillus*, *Listeria*, *Staphylococcus*, *Citrobacter* (Apun *et al.*, 1999) and *Edwardsiella* (Seong Wei *et al.*, 2011).

To date, repetitive element polymerase chain reaction (rep-PCR) has become one of the highly powerful molecular tools applicable for the identification of bacteria and differentiation of bacterial strains of the same species (Gomez-Gil *et al*., 2004). Although there are different methods of rep-PCR, $(GTG)_5$ has proved to show the highest discriminatory power (Mohapatra *et al.*, 2007; Gevers *et al*., 2001) and effective in screening a large amount of bacterial strains (Gevers *et al*., 2001). In addition, $(GTG)_5$-PCR is particularly useful for identification and intraspecies differentiation of bacterial genomes (Gevers *et al.*, 2001). In fact, $(GTG)_5$ PCR is a type of repetitive extragenic palindromic (rep)-PCR that amplifies the $(GTG)_5$ repetitive element that lays throughout the bacterial genomes. Apart from high discriminatory power and high-throughput of strains, this simple PCR-based method also comes with the advantages that it is a low cost and reliable tool for typing a broad range of Gram-negative bacteria and a narrow range of Gram-positive bacteria (Versalovic *et al.*, 1994; Olive & Bean, 1999; Gevers *et al.*, 2001).

Considering the need to contribute more detailed data about the bacteria identities in Sarawak aquaculture, the main intention of this study is to determine and to group the bacteria based on genetic differences through $(GTG)_5$-PCR analysis, and then identify their species based on the 16S rRNA gene sequencing.

Materials and Methods

Samples collection and processing

Sampling was carried out at aquaculture farm located at Miri, Limbang and Lundu. Three types of samples collected were sediment, water and cultured species. The sediment and water samples were taken using a sterile Polyvinyl chloride (PVC) pipe and 100 ml sterile Schott's bottle, respectively (Huys, 2003). *Litopenaeus vannamei* or Pacific White Shrimp (local name: *udang putih*) was freshly caught from the farms at Lundu and Limbang. *Pangasius pangasius* or River Catfish (local name: *ikan patin*) was freshly

*Corresponding author.
Email: *kathmichelle@hotmail.com*